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Reprinted from the Journal of Physiology.
Vol. XII. No. 1, 1891.

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MYOSIN-PEPTONE. By R. H. CHITTENDEN, *Professor of Physiological Chemistry*, AND RALPH GOODWIN, Jr., *Ph.B.*

(*Contributions from the Sheffield Biological Laboratory of Yale University.*)

In the digestion of myosin from muscle tissue with alkaline pancreatic juice there invariably remains a large residue of insoluble matter, which resists the solvent action of trypsin. That portion of the myosin which is dissolved is quickly converted into peptone and crystalline products, the digestive fluid on saturation with ammonium sulphate giving little or no precipitate of myosinoses. As is now well known, alkaline trypsin solutions convert all proteids into true peptones far more readily than pepsin-acid solutions, the real difference in action apparently lying in the fact that pepsin changes the first formed proteoses slowly, whereas trypsin acts with the same energy upon the proteoses as upon the original proteid. Myosin, however, is peculiar in that the digestible portion of it is so readily convertible into peptone by trypsin; myosinoses are first formed as in the digestion with pepsin, but under the action of trypsin they quickly give place to true peptone, the hemi-portion of which is in turn quickly broken down into crystalline products. It has been the object of the present study to ascertain the nature and composition of the peptone thus formed, and incidentally also the character of the insoluble matter which is such a prominent feature in the digestion of myosin with pancreatic juice.

The myosin used in the preparation of the several samples of peptone was prepared at different times from ox muscle by soaking the thoroughly washed and finely divided tissue in 5 per cent. solution of ammonium chloride, and then separating the dissolved myosin by dialyzing the filtered solution in running water until the chloride was entirely removed[1]. The semi-gelatinous myosin was then filtered,

[1] See "Myosin und Myosinosen." Kühne and Chittenden. *Zeitschrift für Biologie*, Bd. xxv., p. 358.

Also "Nature and chemical composition of the myosin of muscle tissue." Chittenden and Cummins. *Studies from Laboratory of Physiological Chemistry, Yale University*, Vol. III., p. 115.

washed with water and transferred directly to a sodium carbonate solution of 0·5 per cent. strength, in preparation for its treatment with trypsin. All treatment with alcohol and ether was avoided as being unnecessary and indeed undesirable, for in a previous study of the myosinoses[1] formed in gastric digestion, it was observed that contact of the myosin with alcohol and ether tended to diminish decidedly its solubility in the digestive fluid.

The ferment solution employed in the digestion of the myosin was prepared from dried ox pancreas according to Kühne's well-known method, and was purified by long-continued dialysis from both an acid and alkaline solution.

Several digestions were made, each with large quantities of pure myosin, four of which were carried to a successful termination.

The details of the digestion were as follows; the myosin extracted from 20—30 pounds of fresh lean beef was placed in 3—5 litres of 0·5 per cent. sodium carbonate, in which it was soon swollen to a jelly and partially dissolved. To this was added an alkaline trypsin solution, prepared from 40 grams of dried ox pancreas, after which the mixture, well thymolized, was warmed at 40° C. with frequent agitation. The ferment quickly began to act upon the gelatinous myosin and the undissolved matter gradually took on a denser appearance. At the end of six days about three-fourths of the myosin was usually dissolved. The undigested residue was then filtered off, washed with water, and again warmed at 40° C. for one week with a fresh and active trypsin solution in 2 litres of 0·5 per cent. sodium carbonate. This treatment, however, did not appear to diminish its amount to any great extent. It was again filtered off, washed with water, and then dissolved in warm 0·2 per cent. hydrochloric acid, from which it was ultimately precipitated by neutralization in the form of heavy flocks. In order to free the substance from any trace of adhering myosin it was warmed at 40° C. for 3—4 days with an active pepsin-hydrochloric acid solution. Neutralization of the acid fluid yielded a heavy precipitate, and examination of the filtrate showed only the presence of traces of cleavage products. From this it was assumed that the antialbumid-like substance was probably as pure as it could be prepared and it was therefore collected, after neutralization of the acid-pepsin solution, on a filter and washed thoroughly with water, and finally with 95 per cent. alcohol and ether.

Its composition, after being dried at 110° C. until of constant weight, was as follows:

[1] Kühne and Chittenden, *loc. cit.*

Myosin-antialbumid.

I. 0·3452 gram substance gave 0·2328 gram H_2O = 7·49 per cent. H and 0·7132 gram CO_2 = 56·34 per cent. C.

II. 0·3162 gram substance gave 0·2143 gram H_2O = 7·53 per cent. H and 0·6501 gram CO_2 = 56·06 per cent. C.

III. 0·2450 gram substance gave 28·3 c.c. N at 16·9° C. and 758·4 mm. pressure = 13·63 per cent. N.

IV. 0·2320 gram substance gave 0·0052 gram ash = 2·24 per cent.

V. 0·9095 gram substance gave 0·0201 gram ash = 2·21 per cent.[1]

VI. 0·4821 gram substance fused with KOH + KNO_3 gave 0·0466 gram $BaSo_4$ = 1·30 per cent. S.

Percentage composition of the ash-free substance.

					Average
C	57·62	57·35	–	–	57·48
H	7·66	7·69	–	–	7·67
N	–	–	13·94	–	13·94
S	–	–	–	1·32	1·32
O					19·59
					100·00

Very noticeable is the high content of carbon and the correspondingly low percentage of nitrogen, myosin itself having an average content of 52·79 per cent. of carbon and 16·86 per cent. of nitrogen. The reactions of the substance were in no ways peculiar, being essentially the same as those of other like proteid bodies, excepting its extreme non-digestibility in both pepsin and trypsin solutions.

The original alkaline fluid containing the soluble products of digestion, after filtration from the undigested myosin and other insoluble matter, was carefully neutralized, without yielding any neutralization precipitate, and then concentrated to a thin syrup. The fluid gave no coagulum on heating, and by saturation of a portion with ammonium sulphate only a very slight indication of myosinoses was obtained. There was, however, a large amount of leucin and tyrosin present, so much indeed that as the fluid cooled a very large crop of crystals separated, composed wholly of the well-known forms of leucin and tyrosin. Bromine water also gave a very strong violet purple colour.

[1] The ash contained only a very slight trace of sulphate.

Considerable leucin and tyrosin were separated from the thick peptone solution by crystallization, after which the peptone itself was precipitated as a gummy mass by a large excess of hot alcohol. The peptone naturally still contained a large amount of leucin and tyrosin. This was removed by boiling the gummy precipitate with large quantities of 95 per cent. alcohol as long as the alcohol gave evidence of the presence of these bodies. This process consumed considerable time and involved considerable loss of peptone owing to the solubility of the latter in weak alcohol. Finally, the peptone remaining was dissolved in water and dialyzed in running water until all soluble salts were removed. This, likewise, aided in the removal of the last traces of leucin and tyrosin. From the suitably concentrated fluid, the purified peptone was again precipitated by hot alcohol, boiled with alcohol several times and finally dried.

Four distinct samples of peptone, representing as many digestions of different lots of myosin, were prepared in this manner, and after being dried at 110° C. until of constant weight were analyzed with the following results:

Myosin-peptone. A.

I. 0·3619 gram substance gave 0·2185 gram H_2O = 6·70 per cent. H and 0·6232 gram CO_2 = 46·95 per cent. C.

II. 0·3835 gram substance gave 0·2290 gram H_2O = 6·63 per cent. H and 0·6614 gram CO_2 = 47·03 per cent. C.

III. 0·3071 gram substance gave 39·5 c.c. N at 14·6° C. and 760·9 mm. pressure = 15·38 per cent. N.

IV. 0·2912 gram substance gave 37·8 c.c. N at 15·4° C. and 760 mm. pressure = 15·45 per cent. N.

V. 0·4364 gram substance gave 0·0216 gram ash = 4·94 per cent.

VI. 0 4042 gram substance gave 0·0196 gram ash = 4·84 per cent.

VII. The ash from 0·8406 gram substance gave 0·0126 gram $BaSo_4$ = 0·20 per cent. S.

VIII. 0·7051 gram substance fused with KOH + KNO_3 gave 0·0693 gram $BaSo_4$ = 1·34 per cent. S; deducting 0·2 per cent. = 1·14 per cent. S.

IX. 0·4231 gram substance fused with KOH + KNO_3 gave 0·0394 gram $BaSo_4$ = 1·27 per cent. S; deducting 0·2 per cent. = 1 07 per cent. S.

Percentage composition of the ash-free substance.

							Average
C	49·36	49·44	–	–	–	–	49·40
H	7·05	6·97	–	–	–	–	7·01
N	–	–	16·17	16·24	–	–	16·20
S	–	–	–	–	1·19	1·12	1·15
O							26·24
							100·00

Myosin-peptone. B.

I. 0·2540 gram substance gave 0·1510 gram $H_2O = 6·60$ per cent. H and 0·4395 gram $CO_2 = 47·19$ per cent. C.

II. 0·3698 gram substance gave 0·1547 gram $H_2O = 6·65$ per cent. H.

III. 0·2631 gram substance gave 0·4558 gram $CO_2 = 47·24$ per cent. C.

IV. 0·3580 gram substance gave 48·3 c.c. N at 17·0° C. and 765·6 mm. pressure = 16·02 per cent. N.

V. 0·4027 gram substance gave 55·0 c.c. N. at 17·0° C. and 760·2 mm. pressure = 16·15 per cent. N.

VI. 0·3629 gram substance gave 0·0137 gram ash = 3·77 per cent.

VII. 0·6373 gram substance gave 0·0231 gram ash = 3·62 per cent.

VIII. The ash from 1·0002 gram substance gave 0·0142 gram $BaSo_4$ = 0·19 per cent. S.

IX. 0·8136 gram substance gave by fusion with $KOH + KNO_3$ 0·0891 gram $BaSo_4 = 1·50$ per cent. S; deducting 0·19 per cent. = 1·31 per cent. S.

X. 0·9188 gram substance gave by fusion with $KOH + KNO_3 = 0·0871$ gram $BaSo_4 = 1·30$ per cent. S; deducting 0·19 per cent. = 1·11 per cent. S.

Percentage composition of the ash-free substance.

							Average
C	49·00	49·05	–	–	–	–	49·03
H	6·85	6·90	–	–	–	–	6·87
N	–	–	16·63	16·76	–	–	16·70
S	–	–	–	–	1·31	1·11	1·21
O							26·19
							100·00

Myosin-peptone. C.

I. 0·3262 gram substance gave 0·1803 gram $H_2O = 6·14$ per cent. H and 0·5262 gram $CO_2 = 43·99$ per cent. C.

II. 0·3282 gram substance gave 0·1796 gram $H_2O = 6·08$ per cent. H and 0·5278 gram $CO_2 = 43·85$ per cent. C.

III. 0·2511 gram substance gave 31·5 c.c. N at 18·9° C. and 758·9 mm. pressure = 14·80 per cent. N.

IV. 0·3626 gram substance gave 0·0410 gram ash = 11·30 per cent.

V. 0·4364 gram substance gave 0·0491 gram ash = 11·25 per cent.

VI. The ash from 0·7790 gram substance gave 0·0375 gram $BaSo_4$ = 0·66 per cent. S.

VII. 0·3162 gram substance fused with $KOH + KNO_3$ gave 0·0415 gram $BaSo_4 = 1·80$ per cent. S; deducting 0·66 per cent. = 1·14 per cent. S.

Percentage composition of the ash-free substance.

					Average
C	49·58	49·45	–	–	49·51
H	6·91	6·85	–	–	6·88
N	–	–	16·68	–	16·68
S	–	–	–	1·27	1·27
O					25·66
					100·00

Myosin-peptone. D.

I. 0·6823 gram substance gave 0·3980 gram $H_2O = 6·48$ per cent. H and 0·1746 gram $CO_2 = 46·94$ per cent. C.

II. 0·3520 gram substance gave 0·2032 gram $H_2O = 6·41$ per cent. H and 0·6059 gram $CO_2 = 46·94$ per cent. C.

III. 0·3829 gram substance gave 0·2189 gram $H_2O = 6·35$ per cent. H and 0·6600 gram $CO_2 = 47·06$ per cent. C.

IV. 0·4621 gram substance gave 0·2721 gram $H_2O = 6·53$ per cent. H and 0·7973 gram $CO_2 = 47·05$ per cent. C.

V. 0·3437 gram substance gave 46·7 c.c. N at 14·7° C. and 762·1 mm. pressure = 16·18 per cent. N.

VI. 0·3320 gram substance gave 44·7 c.c. N at 14·7° C. and 766·4 mm. pressure = 16·18 per cent. N.

VII. 0·5608 gram substance gave 0·0255 gram ash = 4·54 per cent.

VIII. 0·6166 gram substance gave 0·0265 gram ash = 4·29 per cent.

IX. The ash from 1·1774 gram substance gave 0·0119 gram $BaSo_4$ = 0·13 per cent. S.

X. 0·8895 gram substance gave by fusion with $KOH + KNO_3$ 0·0753 gram $BaSo_4 = 1·16$ per cent. S; deducting 0·13 per cent. = 1·03 per cent. S.

XI. 0·9148 gram substance gave by fusion with $KOH + KNO_3$ 0·0746 gram $BaSo_4 = 1·12$ per cent. S; deducting 0·13 per cent. = 0·99 per cent. S.

Percentage composition of the ash-free substance.

									Average
C	49·04	49·04	49·17	49·15	–	–	–	–	49·10
H	6·77	6·70	6·53	6·82	–	–	–	–	6·70
N	–	–	–	–	16·90	16·90	–	–	16·90
S	–	–	–	–	–	–	1·03	1·00	1·01
O									26·29
									100·00

The composition of the four products is seen to be essentially the same, for considering the nature of the substance and the extreme difficulty in purifying it the slight differences between the individual bodies have little significance. In fact, the agreement in composition is such as to constitute a reasonable guarantee of purity. The most noticeable feature in the composition of the peptone is the low percentage of carbon, while comparison of its composition with that of myosin and the myosinoses[1] makes it plainly evident that the peptone is formed by the further hydration of deuteromyosinose.

	C	H	N	S	O
Myosin	52·79	7·12	16·86	1·26	21·97
Protomyosinose	52·43	7·17	16·92	1·32	22·16
Deuteromyosinose	50·79	7·42	17·00	1·22	23·39
Myosin-peptone[2]	49·26	6·87	16·62	1·16	26·09

Hence, in myosin-peptone we have another illustration of the fact that peptones differ widely from the mother proteid in containing a much lower percentage of carbon[3]; a fact, which lends decided favour to the view that the formation of peptone is the result of a process of hydration.

In reactions, myosin-peptone does not differ materially from other peptones equally free from albumoses. Like the peptones previously described by Kühne and Chittenden[4] it is characterized mainly by lack of precipitation with the ordinary reagents; acetic acid and potassium ferrocyanide, cupric sulphate, lead acetate, etc., giving only

[1] Myosinoses formed in gastric digestion. Kühne and Chittenden, *loc. cit.*

[2] Average of the four products.

[3] "Ueber die Peptone." W. Kühne and R. H. Chittenden. *Zeitschrift für Biologie*, Bd. XXII., p. 452.

[4] "Ueber die Peptone," p. 450.

a slight turbidity, and this was doubtless due in part to the adhering mineral salts which could not be wholly removed.

Boiled with potassium hydroxide and lead acetate an aqueous solution of the peptone becomes somewhat darker in colour, but without any pronounced separation of lead sulphide.

Boiled with concentrated hydrochloric acid there is a noticeable darkening of the fluid. Millon's reagent gives a heavy white precipitate, which on boiling takes on a dirty yellow colour, then reddish, while the fluid has a dirty red appearance.